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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/812,707	LIEW, CHOONG-CHIN	
	Examiner	Art Unit	
	Juliet C. Switzer	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 November 2007 and 08 January 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *49,50,52,53 and 56-79* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *49, 50, 58-79* is/are rejected.

7)☒ Claim(s) *52, 53, 56, and 57* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. This action is written in response to applicant's correspondence received 11/13/07 and 1/8/08. Claims 49, 50, 52, 53, 56, and 57 have been amended, claims 1-48, 51, 54, and 55 have been canceled, and claims 58-79 have been added. Claims 49, 50, 52, 53, 56, and 57-79 are pending are examined herein. Applicant's amendments and arguments have been thoroughly reviewed, but are not persuasive to place the claims in condition for allowance for the reasons that follow. Any rejections not reiterated in this action have been withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

This action is FINAL.

2. The declaration filed 11/13/07 and again 1/8/08 has been considered and is discussed in the response to remarks section.

Claim Objections

3. Claims 52, 53, 56, and 57 are objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim cannot depend from another multiply dependent claim. Each of these multiply dependent claims depends from multiply dependent claim 60. See MPEP § 608.01(n). Accordingly, the claims not been further treated on the merits.

4. The gene referred to in the instant specification and claims as TNFRSF7 is also known in the art as CD27.

Claim Rejections - 35 USC § 102

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

6. Claims 58, 60, 61, 62, 63 , 71, 72, and 73 are rejected under 35 U.S.C. 102(e) as being anticipated by Cocks et al.

Cocks et al. teach methods for analyzing a sample using a collection of genes implicated in blood cell biology, and the collection includes TNFRSF7 (throughout; see SEQ ID NO: 1027 in Table 1).

Cocks et al. teach a method for analyzing body fluid samples, including blood samples (Col. 10, lines 54-58), wherein RNA is isolated from the samples, the target polynucleotides are reverse transcribed into cDNA, a DNA is amplified from that cDNA (Col. 11, line 1 and following) and the cDNA is then hybridized to a collection of polynucleotides which include TNFRSF7 (Col. 12-13 and throughout). Thus, Cocks et al. teach a method for detecting expression of TNFRSF7 in a human test subject comprising detecting RNA encoded by said gene in a blood sample of said test subject, using an oligonucleotide of predetermined sequence which is specific for RNA encoded by TNFRSF7.

The method taught by Cocks et al. includes quantifying a level of RNA encoded by said gene in a sample (Col. 13, lines 4-25) and comparing said level of RNA to a quantified level of control (Col. 13, lines 11-20 and Col. 6 lines 54-65). Control subjects taught by Cocks et al. include healthy patients (Col. 6, beginning at line 55).

Cocks et al. teach quantifying relative to "normalization genes" which are housekeeping genes within the scope of the claim (see Col. 13).

Claims 71-73 are rejected because Cocks et al. teach measuring expression in blood samples. In order to do so, a whole blood sample inherently would have to be taken from an individual. While Cocks et al. are silent as to how the RNA will be isolated or if the cells will be fractionated, the instant claims are drawn using "comprising" language and allow for additional manipulations of the whole blood sample which are not expressly set forth in the claims.

4. Claims 58, 59, and 71 are rejected under 35 U.S.C. 102(b) as being anticipated by Orengo et al. (Clin Exp Immunol. 1997: 107:608-613).

Orengo et al. teach a method which includes taking peripheral blood samples from volunteers and detecting therein expression of CD27 by RT-PCR using CD27 gene specific primers- that is primers that are of predetermined sequence and are specific only for RNA encoded by CD27 (which is also known as TNFRSF7; p. 608-609). Orengo et al. isolated lymphcytes, but this inherently would have occurred from a whole blood sample. Thus "the blood sample" of the subjects was a whole blood sample. The claims are drawn using "comprising" language which is permissive of additional steps other than those specifically set forth, in this case steps to isolate the lymphocytes.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 59, 60, 61, 62, 63 , 71, 72, and 73 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cocks et al. (US 6607879) in view of Chenchik et al. (US 5,994,076).

The teachings of Cocks et al. have been discussed previously in this office action.

Regarding claim 59, Cocks et al. do not teach a method which includes producing an amplification product from RNA encoded by said gene using primers specific for only for RNA encoded by said gene and/or for cDNA complementary to RNA encoded by said gene.

However, at the time the invention was made, it was known to use gene specific primers to produce amplification products prior to hybridization with predefined arrays, as taught by Chenchik et al. (throughout; Col. 11).

It would have been prima facie obvious to one of ordinary skill in the art to have modified the invention taught by Cocks et al. so as to have used gene specific primers to amplify target sequences prior to hybridization with a microarray. In this case, all of the claimed elements were known in the prior art and one skilled in the art could have combined the known elements as claimed to provide a predictable result, namely the production of probe hybridization molecules particularly amplified to hybridize to the array taught by Cocks et al.

Claims 60, 61, 62, 63 , 71, 72, and 73 are included in this 103 to address the embodiment wherein they depend from claim 59.

7. Claims 58, 60, 61, 62, 63, 71, 72, 73, 75, 76, 77, and 78 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thykjaer et al (Cancer Research, 61, 2492-2499, March 15, 2001) in view of Cocks et al. and Sharma et al. (WO 98/49342, as cited in IDS).

Thykjaer et al. teach a method for detecting expression of a variety of human genes in human test subjects diagnosed of having bladder comprising detecting RNA encoded by said

gene in said subject using an oligonucleotide of predetermined sequence which is specific for RNA encoded by said gene and/or for cDNA complementary to RNA encoded by said gene. In particular, Thykjaer et al. teach differential expression anlaysis of bladder tumor cells relative to healthy cells using microarray analysis (p. 2493).

Thykjaer et al. do not teach applying their analysis to the gene expression in a blood sample, and in particular do not teach detecting TNFRSF7 in a blood sample.

Cocks et al. teach methods for analyzing a sample using a collection of genes implicated in blood cell biology, and the collection includes CLK1 (throughout; see SEQ ID NO: 699 in Table 1).

Cocks et al. teach methods for analyzing a sample using a collection of genes implicated in blood cell biology, and the collection includes TNFRSF7 (throughout; see SEQ ID NO: 1027 in Table 1).

Cocks et al. teach a method for analyzing body fluid samples, including blood samples (Col. 10, lines 54-58), wherein RNA is isolated from the samples, the target polynucleotides are reverse transcribed into cDNA, a DNA is amplified from that cDNA (Col. 11, line 1 and following) and the cDNA is then hybridized to a collection of polynucleotides which include TNFRSF7 (Col. 12-13 and throughout). Thus, Cocks et al. teach a method for detecting expression of TNFRSF7 in a human test subject comprising detecting RNA encoded by said gene in a blood sample of said test subject, using an oligonucleotide of predetermined sequence which is specific for RNA encoded by TNFRSF7.

The method taught by Cocks et al. includes quantifying a level of RNA encoded by said gene in a sample (Col. 13, lines 4-25) and comparing said level of RNA to a quantified level of

control (Col. 13, lines 11-20 and Col. 6 lines 54-65). Control subjects taught by Cocks et al. include healthy patients (Col. 6, beginning at line 55).

Cocks et al. teach quantifying relative to "normalization genes" which are housekeeping genes within the scope of the claim (see Col. 13).

Cocks et al. do not particularly and clearly point out that the RNA tested is total blood RNA, an embodiment set forth in many of the claims in this application.

Sharma et al. teach that from the very early stages of diseases the whole organism response to the changed condition (p. 10, 4th full ¶). In light of this, Sharma et al. teach a method for identifying a marker useful for diagnosing a disease comprising the steps of detecting the presence of RNA in an unfractionated sample of whole blood from each of one or more subjects having said disease and quantifying a level of said RNA in said sample. Namely, Sharma et al. teach the preparation of gene transcript patterns beginning with extraction of mRNA from tissues, cells or body parts of an individual or organism which has a disease or condition (p. 7, final ¶, p. 12, 1st ¶), and particularly teach the isolation of mRNA from unfractionated whole blood samples, where unfractionated is interpreted as meaning that the cell types within blood were not separated from one another (p. 35, section 5.1.1). Sharma et al. teach quantifying the level of expression and determining a difference between the quantified level in the sample from the diseased subject and a similarly quantified level of genes of control RNA from an unfractionated sample of whole blood from each of one or more first control subjects (p. 5, step (d); p. 15, first full ¶; p. 18, step (f); p. 11, final ¶). Sharma et al. teach that these methods are carried out by producing amplification products from RNA extracted from an unfractionated sample of whole blood (p. 18 and p. 35, Example 5). Sharma et al. specifically

suggest that this method can be applied to the study of a variety of different types of cancer (p 6, 2nd ¶).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Thykjaer et al. so as to have additionally tested the blood of the patients having bladder cancer and the healthy control samples using the microarray taught by Cocks et al., and in particular to have completed this testing on total blood RNA. One would have been so motivated by the express teachings of Sharma et al. that disease exerts a global effect on individuals and that this effect can be measured by gene expression in the blood. The identification of markers for disease in the blood suggests a potential minimally invasive method to detect bladder cancer. One would have been motivated to use the microarray analysis taught by Cocks et al. since they teach that their array has potential use in the identification of genes differentially expressed in cancers.

At the time the invention was made, in differential expression assays using microarrays, expression statistical significance indicating a difference between two expression levels was commonly met if the p value was <0.05. Under this assumption, the values set forth in claims 75 and 76, if observed would when practicing the method taught by Thykjaer et al. in view of Cocks et al. and Sharma would have been be considered to indicate differentially expressed genes by one of ordinary skill in the art at the time the invention was made. Furthermore, at the time the invention was made, it was routine to consider differences of expression of at least three fold or more to be indicators of differentially expressed genes.

Claim Rejections - 35 USC § 112

8. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

9. Claims 64-79 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a rejection for new matter.

10. Claim 64 appears to have new matter. The specification does not provide basis for a claim which broadly states that any time a test subject's RNA expression of TNFRSF7 is "lower" than the expression of healthy control subjects that the subject is a candidate for having bladder cancer. Regarding the expression of TNFRSF7, the specification provides only very limited data, while this claim specifically recites "lower" expression values. The specification teaches in table 3J identified that TNFRSF7 is differentially expressed between a group of patients having bladder cancer and healthy controls. Example 20 similarly provides 3,518 genes that were identified as being differentially expressed between bladder cancer patients and non bladder cancer individuals, and TNFRSF7 is also among these. The tables list genes that were differentially expressed, but does not provide any further information. For example, the tables do not teach if the expression was higher or lower in bladder cancer patients versus controls. Thus, the statement in the claim regarding classifying the subject as a candidate for bladder cancer if the RNA level "is lower" appears to be new matter.

Furthermore, claims 65, 69, and 75 also have new matter because they not only recite that the expression is lower, which is problematic for the previously stated reasons, but even more specifically states that it is at least 3 times lower, for which no basis has been identified in the specification.

Likewise, claims 66, 70, and 76 have new matter because they recite that the level is 3.2 times lower than that of the control subjects and no basis has been identified in the specification for this value.

In claims 68, 70, and 76 the limitation "with a p value equal to 0.04" appears to be new matter. Applicant did not identify basis for this limitation in the response and the examiner was unable to identify basis for the limitation.

Claim 79 has new matter because it recites determining a "statistically lower" and "statistically higher" levels of TNFRSF7 expression than levels in particular controls. As previously discussed, the specification does not provide basis for levels of TNFRSF7 expression that are statistically higher or lower, because while the specification teaches there is a difference in expression between bladder cancer samples and healthy control samples, the specification is silent as to the directionality of the difference.

11. In claims 71, 72, 73, 74, and 77, the limitation that the blood samples "comprises leukocytes which a have not been fractionated into cell types" is new matter. Such a recitation includes, for example, testing a blood sample where the red blood cells and the white blood cells have been separated, and also includes, the testing of whole blood RNA. There is clearly basis for the latter, but not the former. Applicant asserts in the remarks that this claim limitation finds clear support in the specification, including figure 5C which shows standardized fractions of

leukocytes. However, these are not leukocytes that have not been fractionated into cell types, as they have clearly been fractionated into cell types. While RNA levels have been determined in each of the fractions, this is not basis for the negative limitation "have not been fractionated into cell types." There is no discussion or example in the specification of the testing of RNA in blood samples which comprise leukocytes which have not been fractionated into cell types. Applicant has attempted to present a claim which excludes a particular process step from a method (that is, fractionating the leukocytes) and then provides basis for the exclusion of the step in a method where the opposite occurred. This is not sufficient basis for the claim limitation because there is nothing in the specification that suggests applicant contemplated the exclusion of a step of fractionating leukocytes into cell types. Therefore, the claims are rejected for having new matter.

All claims which depend from the specifically discussed claims are rejected for having new matter because of their dependency from the specifically enumerated claims.

12. Claims 49, 50, 58-74, and 79 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Nature of the invention

Claim 49 is drawn to a method detecting bladder cancer disease in a human test subject, and feature a step of quantifying a level of RNA encoded by an TNFRSF7gene in a blood sample from a single test subject and comparing the level with a quantified level of RNA encoded by said gene in blood samples from control subjects who are classified as healthy control subjects,

and comparing the level of RNA in the sample with control subjects who are classified as having bladder cancer. Claim 49 sets forth that a determination of a statistically significant similarity between the test level and the level of control subjects having said bladder cancer and a statistically significant difference between the test level and the level of healthy control subjects "is indicative of said bladder cancer."

The nature of the invention requires the knowledge of a reliable association between comparing TNFRSF7expression and the indication that bladder cancer is present in a human. Further, the practice of the invention requires an understanding of how the presence of bladder cancer effects the level of TNFRSF7expression in human blood.

Claim 58 is drawn to a method for detecting expression of a gene encoding a TNFRSF7in a human "test subject." Claims which depend from claim 58 set forth that the detected expression is quantified and compared to quantified level of control RNA encoded by said gene in blood samples of control subjects. Listed control subjects include healthy subjects and subjects having bladder cancer. Further dependent claims set forth steps of classifying or identifying the test subject as being a candidate for having bladder cancer depending on the outcome of the comparing steps. Thus, it is clear that the intended use of claim 58 and those that depend from claim 58 is for classifying or identifying the test subject as being a candidate for having bladder cancer. Claims 64, 65 and 66 recite that if the level of expression is "lower" than healthy control subjects, then the individual is a candidate for bladder cancer, with claims 65 and 66 reciting at least 3 times lower and 3.2 times lower.

Independent claim 69 sets forth a method for screening a human test subject for having bladder cancer and includes similar detection, quantification, and comparing steps, reciting that a

test subject is a candidate for having bladder cancer if said level of RNA encoded by said gene in said blood sample of the test subject is "at least 3.2 times lower" than that of said healthy control subjects with a p value <0.05. Claim 70 is similar, but recites that the subject is a candidate for having bladder cancer if the level of RNA encoded by said gene is 3.2 times lower than that of said control subjects classified as healthy subjects with a p value equal to 0.04.

The nature of the invention requires the knowledge of a reliable relationship between TNFRSF7expression in blood and the presence of bladder cancer.

In claim 79, the invention is drawn to a method a method for classifying TNFRSF7gene expression in a human, and sets forth steps of quantifying a level of RNA encoded by an TNFRSF7gene, comparing that level to a level of RNA found in blood samples from control subjects having bladder cancer and also comparing it to control subjects who are healthy. The independent claim states that based on particular determinations, the classification of TNFRSF7gene expression results either with that of said subjects having bladder cancer or with that of subjects who are healthy.

The nature of the invention requires the knowledge of a reliable association between TNFRSF7expression and the ability to classify a particular individual's expression with the expression of subjects having bladder cancer or not having bladder cancer, and further, the use of this method requires that there is an underlying assumption that this classification is meaningful. Reading the claims in light of the specification it is clear that applicant intends to use such a classification method in order to provide a tool that is used as part of a diagnostic process, and such a use requires the knowledge of a reliable association underlying the classification. Further, the practice of the invention requires an understanding of how the presence of TNFRSF7affects

the level of TNFRSF7expression in human blood in patients having bladder cancer versus patients that do not have bladder cancer but may have some other disorders.

Many of the claims additionally require a step of comparing the level of RNA detected in a test subject to "a quantified level of control RNA encoded by said gene in blood samples of control subjects." To practice these claims, it is essential to know the quantified level of control RNA encoded by said gene in blood samples of control subjects.

Scope of the claims

Many aspects of the claims remain quite broad.

In some claims the health status of the control individuals is entirely undefined, and encompass subjects with bladder cancer, healthy patients, patients with some other disease, such as lymphoma, patients with a particular stage of bladder cancer, etc.

Some of the claims are very broad in scope because they encompass that ANY level and direction of difference in gene expression between the healthy controls is indicative of said bladder cancer, if that difference is "statistically significant." That is, the claims do not set forth that one level should be higher or lower than the other, and further do not set forth how much of a "difference" between two individuals would be necessary to draw the conclusions set forth in the claims. Some claims recite that a difference is identified but do not require that the difference is statistically significant at any particular level, and so, any level of difference observed can result in classifying the test subject as a candidate for disease. These claims do not recite a level of statistical significance that is required to be reached, and so, the claims remain quite broad since no particular level is required, and the claims even encompass using different levels of statistical significance for different comparisons. The phrase "statistically significant"

describes a mathematical measure of difference between groups, not a particular level of difference which is acceptable. There is no universally accepted level of "statistically significant."

Claim 70 is representative of the narrowest claims set forth in the instant claim set, but the relationship set forth in this claim is not supported by the specification, as noted in the new matter rejection in this office action. This claim specifically defines the control population as healthy subjects and sets forth a very particular ratio of gene expression in the test subject relative the healthy control subjects.

Teachings in the Specification/Examples

Regarding bladder cancer, the specification provides example 19 wherein gene expression profiles of blood samples from individuals having bladder cancer were compared with normal individuals, that is healthy patients. The specification teaches that 4,228 genes were identified as being differentially expressed, and regarding the instant claims, table 3J provides a list of these genes (Example 19). TNFRSF7 is among the genes. Example 20 similarly provides 3,518 genes that were identified as being differentially expressed between bladder cancer patients and non bladder cancer individuals, and TNFRSF7 is also among these.

The tables list genes that were differentially expressed, but does not provide any further information. For example, the tables do not teach if the expression was higher or lower in bladder cancer patients versus controls. Some claims being currently examined set forth that the expression is “lower” with some reciting particular levels, but these limitations are not supported by data in the specification.

The specification does not provide any guidance as to the level of "difference" that is sufficient (1 fold, 2 fold, etc) to result in a conclusion that bladder cancer is detected, nor does the specification provide any guidance as to the direction of the difference (higher or lower expression) that is expected to be observed for any single pairing of samples.

The specification fails to provide information about an essential aspect of the invention, namely, the nature of the difference in expression that was observed between bladder cancer patients and healthy patients. Furthermore, though the specification teaches that this gene is differentially expressed in bladder cancer patients versus healthy patients, the specification teaches this is true for thousands of genes. There is no guidance or analysis of data in the specification to suggest that this gene in particular is sufficient to conclude that bladder cancer is present in a sample, as is instantly claimed. This information is essential to understanding and practicing the claimed invention because it is critical to knowing how to interpret a particular comparison result.

State of the Prior Art and Level of Unpredictability

The expression of genes in examples 19 and 20 was tested by hybridization of samples to a microarray that contains genetic information for tens of thousands of genes. This technology area is highly unpredictable, and as a result significant guidance is required to practice inventions using this type of data. Lee (Clinical Chemistry, 47:8, 1350-1352 (2001)) teaches that despite the technical accuracy of individual observations on an array, these data "are much more prone to numerous false-positive findings fundamentally because of (a) an extremely large number of observations and (b) a very wide dynamic range of gene expression values obtained from gene chip experiments." In view of these unpredictable aspects of applying such data, Lee teaches

that replication is necessary to begin to screen out false positive results. There is no replication in the instant specification.

Observing differences in expression between two populations is a highly unpredictable endeavor. For example, while the specification demonstrates that this gene was differentially expressed in the samples collected, the specification does not undertake analysis to see if this gene is differentially expressed in the blood of patients having other types of cancer or other bladder diseases. Showe et al. teach that TNFRSF7 is a gene useful for detecting cutaneous T-cell lymphoma (CTLC) in unpurified PMBC (¶0108 and Table 1, p. 11). Thus, if one were to detect expression of TNFRS7 in blood that is different from healthy patients, it would be highly unpredictable if this difference is due to the presence of bladder cancer in particular or some other disease or condition. It is highly unpredictable how would one begin to know if that level of expression indicated bladder cancer, CTLC, both, one but not the other, something in between or even some other condition or disorder for which the expression profile has not yet been determined.

Furthermore, although TNFRSF7 was not observed to be differentially expressed in any of the other examples in this specification, it is unknown and unpredictable whether it would be expressed in the blood of patients having other bladder diseases or other cancers or any other diseases which were not tested in the instant specification or diseases which were tested in the instant specification but in a different population of test subjects, and whether this expression would be different from levels of expression in healthy controls. A method for detection which relies on a comparison between expression in the blood of a test subject and control subjects requires the knowledge of this information in order to reliably "detect" bladder cancer, as set

forth in the claims. The instant specification has not established that all difference, no matter the magnitude nor the direction, relative to any control subjects or even relative to a healthy control subject is indicative of bladder cancer. It is not known under what circumstances the result observed in the instantly examined control and test populations would be repeatable, as the results have not been validated. But even if one were to obtain the same result, it would be unknown because applicant did not disclose the magnitude of difference in expression between bladder cancer patients or controls, nor did applicant disclose the direction of variation. All of these inquiries are particularly important in this case since the specification is silent as to which differential expression observations would be sufficient to detect the presence of bladder cancer.

In the post-filing art, Osman et al. provide an analysis which includes microarray hybridization of test and control isolated from total cellular RNA where the test is patients with bladder cancer and the control is healthy individuals (Osman et al. Clinical Cancer Research 2006; 12(11) 3371-3380). Osman et al. teach that 1,088 genes were differentially expressed, and that one of these was TNFRSF7 (Results). Osman et al. teach, in the post filing date art, what the instant specification fails to teach, that is that this gene was underexpressed in bladder cancer patients compared with healthy controls. Osman et al. suggest the use of this gene as part of a panel of expressed genes for detecting bladder cancer, but they do not teach that this gene alone is sufficient to detect bladder cancer. Even in view of this disclosure, Osman et al. teach that their study has several limitations including that "the expression profiles may represent the activation of specific immunologic response to the presence of bladder tumors, and that the profiles identified in this study may be intrinsic to the cohort of patients evaluated in this study

(p. 3379)." The field remains highly unpredictable years after the filing of the instant application, even with the significantly more guidance given in this post-filing date reference.

Further, the claims of the instant application set forth the comparison of the gene expression in a single individual versus as few as two other individuals, and they set forth that a comparing gene expression between the two is "indicative of" bladder cancer. Neither the specification nor the claims set forth a threshold of difference between an individual's expression and the control expression of TNFRSF7 in the blood that would be sufficient to conclude that the difference in gene expression between a test individual and any type control group is "indicative of" any of the recited bladder cancer. Because the claims encompass any level of altered gene expression, it is relevant to point out that the art of Cheung et al (2003) teaches that there is natural variation in gene expression among different individuals. The reference teaches an assessment of natural variation of gene expression in lymphoblastoid cells in humans, and analyzes the variation of expression data among individuals and within individuals (replicates) (p.422, last paragraph; Fig 1). The data indicates that, for example, expression of ACTG2 in 35 individuals varied by a factor of 17; and that in expression of the 40 genes with the highest variance ratios, the highest and lowest values differed by a factor of 2.4 or greater (Fig 3). It is thus unpredictable as to whether or not any level of altered gene expression is indicative of a bladder cancer or the absence of bladder cancer.

The unpredictability of correlating gene expression level to any phenotypic quality is taught in the post-filing art of Wu (2001). Wu teaches that gene expression data, such as microarray data, must be interpreted in the context of other biological knowledge, involving various types of 'post genomics' informatics, including gene networks, gene pathways, and gene

ontologies (p.53, left col.). The reference indicates that many factors may be influential to the outcome of data analysis, and teaches that expression data can be interpreted in many ways. The conclusions that can be drawn from a given set of data depend heavily on the particular choice of data analysis. Much of the data analysis depends on such low-level considerations as normalization and such basic assumptions as normality (p.63 - Discussion). The art of Newton et al (2001) further teaches the difficulty in applying gene expression results. Newton et al. teaches that a basic statistical problem is determining when the measured differential expression is likely to reflect a real biological shift in gene expression, and replication of data is critical to validation (p.38, third full paragraph). There is no replication of data in the instant specification.

Quantity of Experimentation

The instant specification does not provide enabling support for the practice of a single embodiment within the claimed invention. In particular, the specification does not provide adequate guidance to appraise one of ordinary skill in the art as to what levels of TNFRSF7 gene expression must be observed to successfully conclude that bladder cancer is present. Further, although the specification teaches there are differences in TNFRSF7 levels in a bladder cancer population versus a control patient population, the specification is silent as to the nature of the "difference" in magnitude or direction. Thus, given the lack of teaching in the specification and the highly unpredictable nature of the technology, an extensive amount of work would be required to practice the claimed invention.

In order to practice the claimed invention, one would have to undertake an extensive amount of experimentation in a highly unpredictable technology area. One would begin by trying to reproduce the results observed in the instant specification to determine if there is a

relative upregulation or downregulation of TNFRSF7 in bladder cancer patients versus healthy control patients, as the specification does not even provide this minimal guidance. Without this knowledge one would not even begin to know how to interpret any results obtained in practicing the claimed methods. For example, consider the comparison of a test result and a control population of healthy individuals. How different from the average level of expression of healthy individuals would the test result have to be to indicate bladder cancer? Would any difference, up or down regulation be indicative of bladder cancer? Or could one indicate bladder cancer and one CTCL? Is TNFRSF7 expressed in the blood of individuals with a disease other than CTCL and bladder cancer? Is this expression also diagnostic of other cancer or other diseases of the bladder or other disorders entirely unrelated to bladder cancer? In order to reliably use a method for detecting bladder cancer, one would first have to answer at least these questions. One would also, however, have to carry out this testing for validation, for it is possible that the result observed in the instant specification is intrinsic to the cohort of patients evaluated in applicant's study. Further, one would have to undertake experimentation to determine difference thresholds required to determine that a patient has or does not have a disease.

As discussed, this art area is highly unpredictable.

Conclusion

The claims include methods which encompass the detection in blood of the expression of TNFRSF7in a test subject and comparing this expression to control subjects, wherein the results of the comparison are "is indicative of bladder cancer." The identification of gene differential expression/disease indication relationships is a highly unpredictable endeavor, requiring extensive experimentation. The specification provides minimal guidance. In light of the factors

discussed, therefore, it is concluded that it would require undue experimentation to practice the claimed invention.

Although some of claims are drawn to a method of "detecting expression" or "classifying expression," and not to diagnosis or identifying increased likelihood of disease or the like, it is critical to understand how the classification can be used in order use the claimed invention. In this case, the specification does not provide sufficient guidance as to how to use the detecting or classification methods other than in methods that are directed towards diagnostic purposes. What is the meaning of classifying expression "with that of subjects having" bladder cancer or with subjects who are healthy? While one could do the method steps as written, thus satisfying the "how to make" aspect of 112 1st paragraph, the specification does not provide sufficient disclosure to satisfy the how to use aspect of the requirement.

The data in the specification is not replicated. As discussed in the rejection, it is established that the technology on which the instant claims is based is a highly unpredictable technology, and in the face of such a high level of unpredictability, replication is necessary before results can be considered sufficient to support claims directed at classifying the gene expression of an individual test subject. Therefore, even this claim, after having considered all of the factors set forth in this rejection, lacks proper enablement.

Response to Remarks

Applicant traverses the rejection for lack of enablement. Applicant traverses the rejection insofar is it applies to the pending claims, beginning on page 12 of the response.

Applicant states that the instant claims recite three clearly defined sets of controls. Not all claims are so limited (see for example claim 61).

Applicants point out that claims which recite that the test subject is a candidate for bladder cancer if the level of RNA encoded by TNFRSF7 is "lower" than that of healthy subjects, requiring in some, but not all cases, a level of statistical significance of $p<0.05$. However, while this is significantly narrower than the previously pending claims, the limitations are new matter and are not enabled by the specification since the specification is silent as to the difference or magnitude of direction which was observed between the population of healthy subjects and subjects with bladder cancer.

Applicant discusses claim 75 on page 14 of the response. This claim is not included in the enablement rejection.

Applicant states that TNFRSF7 is indeed sufficient to provide an indication of bladder cancer on the grounds that the specification discloses that RNA encoded by the TNFRSF7 gene in a blood sample for a bladder cancer patients is differently expressed relative to healthy subjects. First, applicant did not show that a statistically different level was observed between a single individual's level of expression and healthy patients, as stated in the response. Further, the instant specification fails to provide a critical piece of information with regard to understanding the relationship between TNFRSF7 expression and bladder cancer. The specification invites one of skill in the art to undertake experimentation to (a) determine the relationship between bladder cancer and TNFRSF7 expression and then to (b) validate that relationship. There is a fundamental absence of information given in the specification. The claims all set forth comparing the test level to "a quantified level of RNA encoded by said gene in blood samples from control subjects…" but the specification does not provide this quantified level, or any quantified level. So, it is left to one of skill in the art to establish what is critical for

the practice of the invention. While the specification may rely on the state of the prior art to help enable the invention, the specification may not rely on the state of prior art to supplement what is critical to the practice of the invention- in this case the quantified levels of control RNA encoded by the gene in the control subjects, no matter which type of control subjects.

Applicant points to the declaration which discloses post-filing validation using quantitative RT-PCR and an independent cohort of healthy control subjects and subjects having bladder cancer. Applicant points out that the declaration clearly shows that RNA encoded by the gene TNFRSF7 is present at a statistically lower level in blood of subjects having bladder cancer relative to healthy control subjects.

The declaration demonstrates that TNFRSF7 has significantly lower expression in bladder cancer patients, but this does not make up for the deficiency in the specification. The experimental results disclosed in the declaration add to the teachings in the specification since they teach that TNFRSF7 RNA have been experimentally shown to be significantly lower in bladder cancer patients relative to healthy controls. The claims all rely on comparison to TNFRSF7 quantified levels that are not given in the specification.

It is not known, and unknowable from the specification if the level of expression in other diseases (such as other cancers or bladder diseases) is the same as that for bladder cancer patients. Likewise, as pointed out in the rejection this gene is differentially expressed in blood of patients with CTLC, and so it is not known if this level is the same or different as those patients with bladder cancer. Some of the claims recite that they are methods for "**detecting**" bladder cancer, and so in order to detect the disease one must be able to put the result into a larger context.

Applicant points out that Showe et al. teaches that TNFRSf7 is unregulated in CTLC relative to controls, and that the declaration and Osman et al. teach that that TNFRSF7 is downregulated in blood of bladder cancers relative to controls. This argument relies on the findings in the declaration and the post-filing reference which were not available at the time of filing. The specification does not teach that TNFRSF7 is downregulated relative to controls, and instantly rejected claims (for example 49 and 50) encompass any direction of difference in blood levels of TNFRSF7. The specification has to be enabling at the time of filing. Following the guidance in the specification one would merely be invited to replicate what applicant has done with a hope to obtain the same result, but no way of knowing if one had done so.

Applicant points out that Showe et al. refers to experimental data from purified PBMC but not in blood which is not fractionated into cell types, as taught in Example 19. However, the claims are sufficiently broad so as to encompass samples that are "whole blood." The claims are drawn using "comprising" language and encompass methods where a whole blood sample is taken and then further purified. Further, there is no evidence of record to support the idea that differential expression would not have been observed in a total blood RNA sample. Based on the teachings of the specification one would not know if the levels in bladder cancer patients and CTLC are the same or different, if they are similar, then following the logic of the claim one could still arrive at a conclusion that bladder cancer is "indicated" or "detected" when a different disease is present.

Applicant states on page 16 that one of skill can reasonably predict with a statistically significant probability that a patient may be a candidate for having bladder cancer based on the teachings of the specification. However, as discussed previously in this office action the

specification fails to disclose information that would be critically to any enabled use of the rejected claims.

Applicant states that the attached declaration disclosed post-filing validation experiments. The declaration provides findings that may or may not replicate the findings referred to in the specification. However, it is not knowable from the teachings in the specification if the data actually replicates the findings in the specification since the specification does not give complete data. It is unknown if the relationship observed in the experiment provided in the declaration is the same as the relationship observed using the microarray analysis. One cannot make this comparison because the data given in the specification are incomplete. Based on the disclosed fact pattern in the instant specification, one could not extrapolate that TNFRSF7 expression is sufficient to "detect" or "indicate" bladder cancer, as set forth in the claims. On cannot readily extrapolate whether or not the level of TNFRSF7 is the same or different in bladder cancer and other diseases. If the levels are the same, it would not be sufficient to show that TNFRSF7 expression is the same as a patient with bladder cancer in order to detect bladder cancer. One cannot readily extrapolate that the observation made in the specification is the same universally and not cohort specific since no specific guidance is given in the specification. One cannot readily extrapolate one could successfully differentiate different types or stages of bladder cancer based on the disclosed data.

Applicant disagrees with Wu that expression data needs to be interpreted in view of other biological knowledge. Wu was relied upon for much more than this simple statement. Wu discusses at length many of the factors that make gene expression analysis unpredictable. Applicant's statement that "differential gene expression which is reproducible, and is correlated

with the state of health or disease of the individual does not necessarily result directly in the state of the disease of the individual" is attorney argument which is not supported by evidence on the record. Even if the changes are a result of downstream effects of the pathogenic process, they are related to the state of disease in the individual. Applicant points out that certain prostate markers were used as biomarkers without an understanding of their function. The examiner is not trying to require an understanding of TNFRSF7 in bladder cancer or any other disease, nor does Wu suggest that such is necessary. The examiner is looking to the specification for adequate guidance for making and using an invention in a highly unpredictable field of endeavor.

Applicant states that the results of Cheung et al. cannot be reliably extrapolated to primary blood samples since Cheung et al. are using cultured cell lines. However, this is irrelevant to the point of Cheung et al. which is that among individuals (in this case cell lines) there is natural variability in gene expression for any particular gene. Attorney arguments are not sufficient to establish that this biological fact is not the case. Applicant further states that to the extent that Cheung et al. could still be considered to suggest that larger populations of diseased and control populations may be useful, this experimentation is routine. However, as previously noted, for the reasons discussed, this is not routine experimentation given the lack of guidance in the specification, the lack of working examples, the high degree of unpredictability in the art area and the other factors discussed. In the absence of the critical disclosure of the specification and the unpredictable nature of the technology, the further experimentation is inventive- applicant has provided one of skill in the art with an invitation to discover the actual relationship between TNFRSF7 expression in the blood and bladder cancer.

The instant situation differs tremendously from In re Angstadt, wherein a large number (forty) examples were provided, only one of which did not work. In In re Angstadt, the court determined that there was sufficient guidance in an unpredictable art. The court further stated, however, that "each case must be determined by its own facts." The facts of this case do not support an enabled use for the claims, for all of the reasons discussed in the rejection. Here, the situation is quite different because the specification does not provide data or guidance sufficient to support the claims of any embodiment of the claimed invention, let alone multiple embodiments.

Conclusion

13. No claim is allowed.

14. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Juliet C Switzer whose telephone number is (571) 272-0753. The examiner can normally be reached on Monday, Tuesday, or Wednesday, from 9:00 AM until 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached by calling (571) 272-0735.

The fax phone numbers for the organization where this application or proceeding is assigned are (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571)272-0507.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as

general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

/Juliet C. Switzer/
Primary Examiner
Art Unit 1634

March 31, 2008